UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/616,659	07/09/2003	Costas D. Maranas	P06367US03	9959

27407 7590 07/06/2010
MCKEE, VOORHEES & SEASE, P.L.C.
ATTN: PENNSYLVANIA STATE UNIVERSITY
801 GRAND AVENUE, SUITE 3200
DES MOINES, IA 50309-2721

EXAMINER	
SKOWRONEK, KARLHEINZ R	
ART UNIT	PAPER NUMBER
1631	

NOTIFICATION DATE	DELIVERY MODE
07/06/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patatty@ipmvs.com

Office Action Summary	Application No. 10/616,659	Applicant(s) MARANAS ET AL.	
	Examiner KARLHEINZ R. SKOWRONEK	**Art Unit** 1631	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE _3_ MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on _28 April 2010_.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) _1-22_ is/are pending in the application.

4a) Of the above claim(s) _6,9 and 15-17_ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) _1-5,7,8,10-14 and 18-22_ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _2/10/10 and 4/28/10_.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

Claim Status

Claims 1-22 are pending.

Claims 6, 9, and 15-17 are withdrawn as being directed to a non-elected species as indicated in the response, filed 07 April 2006, to the Office Action dated 20 March 2006.

Claims 1-5, 7-8, 10-14, and 18-22 have been examined.

Claims 1-5, 7-8, 10-14, and 18-22 are rejected.

Priority

The instant application claims priority to provisional application No. 60/395,763, filed 10 July 2002; provisional Application No. 60/417,511, filed 9 October 2002; and Provisional Application No. 60/444,933, filed 3 February 2003.

Claim Rejections - 35 USC § 101

Response to Arguments

The rejection of claims 1, 3-5, 7-8, 10-14, and 18-22 as directed to non-statutory subject matter is withdrawn in view of the amendments to the claims.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

The following rejection is necessitated by amendment.

Claims 1-5, 7-8, 10-14, and 18-22 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 and 19 are unclear with respect to the term “simultaneously”. The metes and bounds are rendered indefinite by the lack in clarity. The claims are directed to methods in which objective functions are combined to generate an optimization problem which is simultaneously solved to identify an optimal solution. The claims recite the phrase “simultaneously solving the linear optimization problem to provide an optimal solution that yields at least one candidate gene”. It is unclear form the claim what applicant intends to encompass. As recited in the claims, a singular optimization problem is optimized. However the use of the term “simultaneously" suggest another operation. The claim provides no indication of the other operation that is to occur "simultaneously". Thus the claim is unclear. If applicant intends to claim that solving the optimization problem simultaneously finds optimal solutions of the objective functions that yield at least a candidate gene deletion, then it is suggested that the claims be amended to reflect such a limitation. Claims 2-5, 7-8, 10-14, 18, and 20-22 are also rejected because they depend from claims 1 and 19, and thus contain the above issues due to said dependence.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

The following rejection is reiterated from the previous action.

Claims 1, 5, 7-8, 10-11, 13-14, and 19-22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hatzimanikatis et al. (IDS entry 2, 8 May 2007), in view of Varma et al. (IDS entry 3, 8 May 2007) and in view of Bhaska et al. (Reviews in Chemical Engineering, Volume 16, Issue 1, p. 1-54, 2000).

The claims are drawn to a method comprising selecting a bioengineering objective function; selecting a cellular objective function forming a linear optimization problem that couples the cellular objective function with the bioengineering objective function; and solving the linear optimization problem to yield a candidate. In some embodiments, the optimization problem includes a binary value for specifying if a flux is active or inactive. In some embodiments, the bioengineering function is over production of a chemical being directed to the relative overproduction of phenylalanine. In some embodiments, the optimization problem includes an uptake constraint. In some embodiments, the performance limits are evaluated on the ability to meet the at least objective function.

Hatzimanikatis et al. shows that objective functions can be formed for any process of interest (p. 1281, col. 2). Hatzimanikatis et al. shows improvements in the product yield, rate of production, and final product concentration are common goals in achieving more efficient and cost-effective bioprocesses (p. 1277, col. 1). Hatzimanikatis et al. shows prior research and industrial practice have clearly shown that very large increases in process performance can be realized by genetic modifications of metabolic control systems (p. 1278, col. 1). Hatzimanikatis et al. shows guidance as to what changes in regulation might be of greatest benefit to improve the network is important (p. 1278, col. 1). Hatzimanikatis et al. shows that objective functions can be formed for any process of interest (p. 1281, col. 2). Hatzimanikatis et al. shows a bioengineering objective function in eqn. 32 relating to the production phenylalanine (p.1284, col. 1). Hatzimanikatis et al. suggests that cellular growth rate

can be defined as an objective function (p. 1278, col. 1). In an embodiment, Hatzimanikatis et al. shows the optimization problem includes a binary value for specifying if a flux is active or inactive (p. 1282, col. 2). In an embodiment, Hatzimanikatis et al. show the bioengineering function is over production of a chemical being directed to the relative overproduction of phenylalanine (p.1284, col. 1). In an embodiment, Hatzimanikatis et al. shows the optimization problem includes an uptake constraint (p.1284, col. 1). In an embodiment, Hatzimanikatis et al. shows the optimization problem includes a stoichiometric constraint (p. 1282, col. 1). In an embodiment, Hatzimanikatis et al. shows the performance limits are evaluated on the ability to meet the at least objective function (p. 1279, col. 1). Hatzimanikatis et al. shows that no improvement in the selectivity for the reference state could be achieved only by enzyme overexpression, without having an effect on the growth rate (p. 1284, col. 2). Thus, Hatzimanikatis et al. suggest growth rate is coupled to amino acid production. Hazimanikatis et al shows a suitably programmed computer for solving an optimization problem (p. 1284, col. 1).

Hatzimanikatis et al. does not show the cellular and bioengineering objective functions that are coupled in a single optimization problem.

Varma et al. shows that bioengineering objective functions and cellular objective functions can be coupled (p. 67, col. 2). Varma et al. shows the mathematical dual of the linear optimization problem has also been evaluated to determine the dual solution (p. 60, col. 1). In figure 2, Varma et al shows a computerized model representing the a plurality of metabolic reactions. An optimal trade-off between growth and biochemical

production can be assessed by choosing a production rate for a particular product between zero and the maximum production rate and then maximizing the growth rate (p. 67, col. 2). Varma et al. shows the balanced growth and biochemical solution shows a higher efficiency compared to a simple addition of the individual solutions (p. 72 col. 2). Varma et al. shows that the balance between growth (cellular objectives) and biochemical production (bioengineering objectives) is important for a successful bioprocess and necessary to provide the backbone of metabolism used to achieve substrate conversion into the desired biochemical (p. 72, col. 1-2).

Bhaskar et al. shows that multiple objective optimization is applied to biochemical engineering problems such as the design of anaerobic digesters (table 1). Bhaskar et al. shows that most real world chemical engineering problems require the simultaneous optimization of several objectives (multi-objective optimization) which cannot be compared easily with each other (are non-commensurate), and so cannot be combined into a single, meaningful scalar objective function (p. 4). Bhaskar et al. shows that objective functions can be coupled through a dual problem such that the dual objective function is always bound to the original objective function called the primal (also known as bi-level)(p. 4-5). Bhaskar et al. shows that if the optimal dual objective function result is identified then the primal objective function result has also been identified (p. 5). The example of the batch reactor of Bhaskar et al. illustrates the combination of objective functions that are analogous to the bioengineering and cellular functions (p. 7-8). The yield of B of Bhaskar et al. analogous to the cellular objective function maximizing growth rate. Bhaskar et al. shows maximization of the yield is

important since it leads to higher amounts of B just as the growth rate of Hatzimanikatis et al. and the biomass of Varma et al. (p. 8). The selectivity of B of Bhaskar et al. is analogous to the bioengineering objective function related to the production of phenylalanine of Hatzimanikatis et al. and product formation of Varma et al. Bhaskar et al. shows maximization of the selectivity is desired since it leads to a reduction in the downstream separation costs (p. 8). Bhaskar et al. show the bi-level optimization programming in a simple problem demonstrates the opposing results of a reaction in which the maximum yield and selectivity of a chemical reaction are sought Bhaskar et al. shows that the between points P and Q both functions approach a maximum at Q. Between Q and R, Bhaskar et al. shows that while selectivity increases, the yield decreases (figure 2). Thus, Bhaskar et al. shows that optimal solutions can be found in divergent objective functions.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the linear programming and objective functions to predict metabolic pathway alterations of Hatzimanikatis et al. with the multi-objective optimization and dual/primal optimization problems of Bhaskar et al. to produce optimization problem that balances the goals of bioengineering and cellular outputs because the technique of bi-level optimization and it's ability to couple objective functions was recognized as part of the ordinary capabilities of one skilled in the art. It would have been further obvious to one of ordinary skill in the art at the time of invention to modify the optimization of Hatzimanikatis et al. by coupling a bioengineering objective function with a cellular objective function as in Varma et al. because Varma et

al. shows that the balance between growth (cellular objectives) and biochemical production (bioengineering objectives) is important for a successful bioprocess and necessary to provide the backbone of metabolism used to achieve substrate conversion into the desired biochemical. One of skill in the art would have been capable of applying bi-level optimization to an optimization problem and the results would have been predictable to one of skill in the art. This is also supported by applicant's statement, “the referenced duality theory concepts were well known to those skilled in the art” (see remarks p.6, filed 31 October 2007). It would have been further obvious to modify the MILP formulation of Hatzimanikatis et al. to express the MILP formulation as bi-level programming problem to identify key enzymes that are capable of regulating or modifying the flux of a metabolism to produce a product because Hatzimanikatis et al. shows metabolite production is coupled to cell growth. One of ordinary skill in the art would have been motivated to find genetic modifications, whether gene deletions or additions, that would allow the maximal production of any commercially relevant metabolite product such that growth rate or some other cellular objective is maximized because an organism having such properties would provide the benefit of higher product yields at lower growth costs.

Response to Arguments

Applicant's arguments filed 28 April 2010 have been fully considered but they are not persuasive. Applicant argues that other than having similar terms in the references, there is nothing in the cited references combination that would lead one skilled in the art to arrive at such a coupling using a linear optimization problem. The argument is not

persuasive. Varma et al shows the computation of the optimal trade-off between simultaneous growth and biochemical production (p. 72, col. 2). As described and claim in the disclosure a bioengineering objective can be biochemical production and a cellular objective can be growth. Varma et al. shows for most of the biochemicals considered here the trade-off is practically linear (p. 72, col. 2). Thus the computation of an optimal trade off between growth and production suggests to one of ordinary skill in the art the coupling of objective functions in an linear optimization problem. Applicant argues Hatzimanikatis et al., in view of Varma et al., in view of Bhaska et al. fail to show or suggest coupling a cellular objective with a bioengineering objective and simultaneously solving for an optimal solution using linear programming. In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., simultaneously solving for an optimal solution using linear programming) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). Applicant argues that the cited references fail to suggest the coupling of divergent objective functions to yield an optimal solution. The argument is not persuasive. Bhaskar et al suggests "Most real-world chemical engineering problems require the simultaneous optimization of several objectives (multiobjective optimization) which cannot be compared easily with each other (are non-commensurate), and so cannot be combined into a single, meaningful scalar objective

function (p. 4). Bahaskar et al shows that Pareto surfaces were also generated for a three objective function problem using linear programming (p. 17).

The following is reiterated from the previous action.

Claims 2, 4, and 18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hatzimanikatis et al., in view of Varma et al., in view of Bhaska et al. as applied to claims 1, 5, 7-8, 10-11, 13-14, and 19-22 above, and further in view of Yang et al.

The claims are drawn to a method comprising selecting a bioengineering objective function; selecting a cellular objective function forming a linear optimization problem that couples the cellular objective function with the bioengineering objective function; and solving the linear optimization problem to yield a candidate. In an embodiment the bioengineering objective function is lactate overproduction and acetate kinase is targeted for deletion. In an embodiment, a bioengineering objective function is underproduction of a chemical. In an embodiment, the candidate is used to modify the organism genetically.

Hatzimanikatis et al., in view of Varma et al., and in view of Bhaska et al. as applied to claims 1, 5, 7-8, 10-11, 13-14, and 19-22 above shows a method comprising selecting a bioengineering objective function; selecting a cellular objective function forming a linear optimization problem that couples the cellular objective function with the bioengineering objective function; and solving the linear optimization problem to yield a candidate.

Hatzimanikatis et al., in view of Varma et al., and in view of Bhaska et al. as applied to claims 1, 5, 7-8, 10-11, 13-14, and 19-22 above does not show an embodiment in which the bioengineering objective function is lactate overproduction and acetate kinase is targeted for deletion.

Yang et al. shows an embodiment in which the bioengineering objective function is lactate overproduction (p. 32, col. 1) and acetate kinase is targeted for deletion (p. 27, col. 1). In an embodiment, Yang et al. shows a bioengineering objective function that is underproduction of a chemical, specifically acetate (p. 27, col. 1). In an embodiment, Yang et al. shows that the candidate is used to modify the organism genetically (p. 32, col. 1). Yang et al. shows the reduction of acetate production is of primary concern in fermentation and recombinant protein production by *E. coli* (p. 26, col. 2). Yang et al. shows that a reduction in acetate production has been shown to enhance recombinant protein production (p. 27, col. 1).

It would have been obvious to one of skill in the art to modify the method of determining gene candidates for alteration in an organism of Hatzimanikatis et al., in view of Varma et al., in view of Bhaska et al. as applied to claims 1, 5, 7-8, 10-11, 13-14, and 19-22 above to include the bioengineering objectives of Yang et al. because Yang et al. shows that a reduction in acetate production has been shown to enhance recombinant protein production is a primary concern in fermentation and recombinant protein production arts.

Response to Arguments

Applicant's arguments filed 28 April 2010 have been fully considered but they are not persuasive. Applicant argues that Yang et al. does not cure the deficiencies of Hatzimanikatis et al., in view of Varma et al., and in view of Bhaskar et al. The argument is not persuasive because Hatzimanikatis et al., in view of Varma et al., and in view of Bhaskar et al. shows a method of identifying modifications to a metabolic pathway by solving a linear optimization problem.

The following rejection is reiterated from the previous action.

Claims 1, 5, 7-8, 10-14, and 19-22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Burgard et al. (Biotechnology and Bioengineering. 2001 74:364-375), in view of Varma et al. (IDS entry 3, 8 May 2007), and in view of Bhaska et al. (Reviews in Chemical Engineering, Volume 16, Issue 1, p. 1-54, 2000).

The claims are drawn to a method comprising selecting a bioengineering objective function; selecting a cellular objective function forming a linear optimization problem that couples the cellular objective function with the bioengineering objective function; and solving the linear optimization problem to yield a candidate. In an embodiment the bioengineering objective function is lactate overproduction and acetate kinase is targeted for deletion. In an embodiment, a bioengineering objective function is underproduction of a chemical. In an embodiment, the candidate is used to genetically modify the organism.

Burgard et al. teach a method of identifying gene candidates for deletion and addition by forming and solving an optimization problem that involves a bioengineering

objective and a cellular objective ("Mathematical modeling of gene additions/deletions", p. 367-369). With respect to the limitation of claim 7, drawn to a candidate deletion and a binary value specifying if a reaction is active or inactive, is also taught by Burgard et al. Burgard et al. teach the use of a binary value to specify if a reaction is active or inactive, "the binary parameter, a_{jk}, is defined to describe which enzymes are coded for by which genes: $a_{jk} = 0$ if gene k has no direct effect on reaction j; 1 if gene k codes for an enzyme catalyzing reaction j ("binary parameter", p367-368, Burgard et al.). This reads on the limitation of claim, the assignment of a binary value to a reaction flux. The limitation of deletions is taught in, "In this study we explore what is the smallest gene set capable of maximizing biomass production on glucose substrate (uptake 10mmol) and what is the maximum number of gene deletions from this gene set that still maintains a specified level of biomass production (p.369)". The above statement also teaches the limitations of claim 13 drawn to the evaluation of performance limits ("smallest gene set"), the limitations of claim 20 and 14, drawn to an objective corresponding to maximizing growth rate, and the limitations of claim 5, drawn to growth ("maximizing biomass production"). The title of Burgard et al. also reads on the limitations of claim 13, performance limits. With respect to the limitations of claim 11, drawn to a chemical uptake constraint, is also taught by Burgard et al., "quantifies the network's uptake (if negative) or secretion (if positive) of metabolite i. (p. 366)" and "stoichiometric coefficient of metabolite i (p.366)". With respect to the limitation of claim 12, drawn to quantifying the cellular objective as an aggregate flux, is also taught by Burgard et al. as "maximized the biomass production flux, $v_{max\ biomass}$. The solution yields the maximum

theoretical level of biomass production ($v_{max\ biomass}$ = 1.25g biomass/gDW·h) achievable by the metabolic network within the stoichiometric constraints (p. 369)". With respect to the limitation of claim 10, drawn to at least one stoichiometric, is also taught by Burgard et al. in "These upper bounds are set by maximizing the given flux n_j subject to the stoichiometric constraints (p. 369)". With respect to the limitations of claims 1 and 19, Burgard et al. shows, "These problems are solved using CPLEX 6.6 accessed via the commercial software package GAMS. Problems with up to 3700 binary variables were solved on an IBM RS6000-270 workstation (p. 369)".

Burgard et al. do not teach the generation of a bilevel optimization problem or the coupling of cellular and biengineering objective functions.

Varma et al. shows that bioengineering objective functions and cellular objective functions can be coupled (p. 67, col. 2). Varma et al. shows the mathematical dual of the linear optimization problem has also been evaluated to determine the dual solution (p. 60, col. 1). An optimal trade-off between growth and biochemical production can be assessed by choosing a production rate for a particular product between zero and the maximum production rate and then maximizing the growth rate (p. 67, col. 2). Varma et al. shows the balanced growth and biochemical solution shows a higher efficiency compared to a simple addition of the individual solutions (p. 72 col. 2). Varma et al. shows that the balance between growth (cellular objectives) and biochemical production (bioengineering objectives) is important for a successful bioprocess and necessary to provide the backbone of metabolism used to achieve substrate conversion into the desired biochemical (p. 72, col. 1-2).

Bhaskar et al. shows that multiple objective optimization is applied to biochemical engineering problems such as the design of anaerobic digesters (table 1). Bhaskar et al. shows that most real world chemical engineering problems require the simultaneous optimization of several objectives (multiobjective optimization) which cannot be compared easily with each other (are non-commensurate), and so cannot be combined into a single, meaningful scalar objective function (p. 4). Bhaskar et al. shows that objective functions can be coupled through a dual problem such that the dual objective function is always bound to the original objective function called the primal (also known as bi-level) (p. 4-5). Bhaskar et al. shows that if the optimal dual objective function result is identified then the primal objective function result is also identified (p. 5).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the linear programming and objective functions to predict metabolic pathway alterations of Burgard et al. with the multi-objective optimization and dual/primal optimization problems of Bhaskar et al. because the technique of bi-level optimization and it's ability to couple objective functions was recognized as part of the ordinary capabilities of one skilled in the art. It would have been further obvious to one of ordinary skill in the art at the time the invention was made to modify the linear programming and objective functions to predict metabolic pathway alterations of Burgard et al. by coupling bioengineering and cellular objective functions as in Varma et al. because Varma et al. shows that the balance between growth (cellular objectives) and biochemical production (bioengineering objectives) is important for a successful bioprocess and necessary to provide the backbone of metabolism used to achieve

substrate conversion into the desired biochemical. One of skill in the art would have been capable of applying bi-level optimization to an optimization problem and the results would have been predictable to one of skill in the art. This is also supported by applicant's statement, “the referenced duality theory concepts were well known to those skilled in the art” (see remarks p.6, filed 31 October 2007). It would have been further obvious to one of ordinary skill in the art to modify the optimization problems of Burgard et al. to identify gene deletions that couples bioengineering, such as metabolite production, and cellular, such as growth rate, objective functions for an organism because Burgard et al. shows that an optimization problem can be formulated to optimize metabolite production and growth and suggests that the optimization can be used to identify gene deletions as well as gene additions. One of ordinary skill in the art would have been motivated to find genetic modifications, whether gene deletions or additions, that would allow the maximal production of any commercially relevant metabolite product such that growth rate or some other cellular objective is maximized because an organism having such properties would provide the benefit of higher product yields at lower growth costs.

Response to Arguments

Applicant's arguments filed 28 April 2010 have been fully considered but they are not persuasive. Applicant argues no suggestion exists to couple objective functions by linear optimization. The argument is not persuasive. Varma et al shows the computation of the optimal trade-off between simultaneous growth and biochemical production (p. 72, col. 2). As described and claimed in the disclosure a bioengineering objective can

be biochemical production and a cellular objective can be growth. Varma et al. shows for most of the biochemicals considered the trade-off is practically linear (p. 72, col. 2). Thus, the computation of an optimal trade off between growth and production suggests to one of ordinary skill in the art the coupling of objective functions in an linear optimization problem. Applicant argues that the cited references fail to suggest the coupling of divergent objective functions to yield an optimal solution. The argument is not persuasive. Bhaskar et al suggests "Most real-world chemical engineering problems require the simultaneous optimization of several objectives (multiobjective optimization) which cannot be compared easily with each other (are non-commensurate), and so cannot be combined into a single, meaningful scalar objective function (p. 4). Bahaskar et al shows that Pareto surfaces were also generated for a three objective function problem using linear programming (p. 17).

The following rejection is reiterated from the previous action.

Claims 1-4, and 18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Burgard et al., in view of Varma et al., and in view of Bhaska et al. as applied to claims 1, 5, 7-8, 10-11, 13-14, and 19-22 above, and further in view of Yang et al.

The claims are drawn to a method comprising selecting a bioengineering objective function; selecting a cellular objective function forming a linear optimization problem that couples the cellular objective function with the bioengineering objective function; and solving the linear optimization problem to yield a candidate. In an embodiment the bioengineering objective function is lactate overproduction and acetate

kinase is targeted for deletion. In an embodiment, a bioengineering objective function is underproduction of a chemical. In an embodiment, a bioengineering objective function is over of a chemical. In an embodiment, the candidate is used to genetically modify the organism.

Burgard et al., in view of Varma et al., and in view of Bhaska et al. as applied to claims 1, 5, 7-8, 10-11, 13-14, and 19-22 above shows a method comprising selecting a bioengineering objective function; selecting a cellular objective function forming a linear optimization problem that couples the cellular objective function with the bioengineering objective function; and solving the linear optimization problem to yield a candidate.

Burgard et al., in view of Varma et al., and in view of Bhaska et al. as applied to claims 1, 5, 7-8, 10-11, 13-14, and 19-22 above does not show an embodiment in which the bioengineering objective function is lactate overproduction and acetate kinase is targeted for deletion.

Yang et al. shows an embodiment in which the bioengineering objective function is lactate overproduction (p. 32, col. 1) and acetate kinase is targeted for deletion (p. 27, col. 1). In an embodiment, Yang et al. shows a bioengineering objective function that is underproduction of a chemical, specifically acetate (p. 27, col. 1). In an embodiment, Yang et al. shows a bioengineering objective function that is underproduction of a chemical, specifically lactate (p. 32, col. 1). In an embodiment, Yang et al. shows that the candidate is used to modify the organism genetically (p. 32, col. 1). Yang et al. shows the reduction of acetate production is of primary concern in fermentation and recombinant protein production by *E. coli* (p. 26, col. 2). Yang et al. shows that a

reduction in acetate production has been shown to enhance recombinant protein production (p. 27, col. 1).

It would have been obvious to one of skill in the art to modify the method of determining gene candidates for alteration in an organism of Burgard et al., in view of Varma et al., and in view of Bhaska et al. as applied to claims 1, 5, 7-8, 10-11, 13-14, and 19-22 above to include the bioengineering objectives of Yang et al. because Yang et al. shows that a reduction in acetate production has been shown to enhance recombinant protein production is a primary concern in fermentation and recombinant protein production arts.

Response to Arguments

Applicant's arguments filed 28 April 2010 have been fully considered but they are not persuasive. Applicants argue Yang et al. does not cure the deficiencies of Burgard et al., in view of Varma et al., and in view of Bhaska et al. as applied to claims 1, 5, 7-8, 10-11, 13-14, and 19-22. The argument is not persuasive because Burgard et al., in view of Varma et al., and in view of Bhaska et al. is not deficient.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to KARLHEINZ R. SKOWRONEK whose telephone number is (571) 272-9047. The examiner can normally be reached on 8:00am-5:00pm Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Marjorie Moran can be reached on (571) 272-0720. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/KARLHEINZ R SKOWRONEK/
Primary Examiner, Art Unit 1631

1 July 2010